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<http://dx.doi.org/10.1016/j.ajpath.2016.05.002>

Authors' Reply

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We thank Meijers et al for their letter and for their interest in our article.¹ Our study tested the hypothesis that the β -galactoside binding animal lectin galectin-3 may play an important role in fibrosis, remodeling, and dysfunction of the pressure-overloaded heart. Using an established mouse model of transverse aortic constriction, we found that galectin-3 was markedly up-regulated in the pressure-overloaded myocardium and localized in macrophages, fibroblasts, and cardiomyocytes. Although cardiomyocyte-specific galectin-3 expression correlated with adverse remodeling and dysfunction, genetic disruption of galectin-3

had no effects on survival, systolic and diastolic dysfunction, or fibrosis, but it delayed the hypertrophic response.

Our findings are consistent with the growing body of evidence supporting the role of galectin-3 as a biomarker in clinical heart failure² and reveal a surprising localization of galectin-3 in a subset of cardiomyocytes. However, our observations are in conflict with the rigorous and well-documented studies by the Lopes-Andres and de Boer laboratories, which suggested that galectin-3 is critically involved in the pathogenesis of cardiac remodeling and fibrotic cardiomyopathy after pressure overload.^{3–5} The authors of the letter argue that the statistically nonsignificant 10% to 20% differences in ejection fraction between groups, observed in male mice, may be important from a clinical perspective. On that basis, they suggest that our findings may support the previously reported beneficial effects of galectin-3 loss in cardiac remodeling. Although we appreciate the comments, we disagree with this interpretation of our findings. Clearly, our data do not support a crucial pathophysiological role for galectin-3 in remodeling and dysfunction of the pressure-overloaded heart. Despite a large sample size (wild-type mice, $n = 50$; galectin-3 null, $n = 46$), galectin-3 null and wild-type mice had comparable survival curves after pressure overload and exhibited no statistically significant differences in end points associated with systolic and diastolic dysfunction. We also did not observe statistically significant effects of galectin-3 loss on myocardial fibrosis. In the absence of statistical significance, the null hypothesis cannot be rejected. Relatively modest and statistically nonsignificant differences in isolated specific end points between groups should not be overinterpreted and likely reflect the variability of responses in animal models.

Considering that all three studies used the same strain of galectin-3 knockout mice, the basis for the disparate findings remains unclear. Sex-specific effects of galectin-3 may be part of the explanation. Previously published studies focused exclusively on male mice,^{3,4} whereas our study examined both male and female animals. Age may also be a factor: in the study by Yu et al,³ young (6- to 10-week-old) mice were used, whereas we studied mature adult animals (2- to 4-month-old) in our experiments. Differences in the severity of pressure load between experimental protocols and in the experimental design may also account for the differences. Although Yu et al³ studied a single time point (28 days after transverse aortic constriction), we performed serial analysis at three different time points, ranging from 7 to 56 days. Considering the growing concerns regarding reproducibility of *in vivo* observations, it is crucial for investigators to report their own experience, especially when the findings contradict published literature. The combined experience from rigorous and well-documented studies from several independent groups paints a more realistic picture than any single study.

Do our findings have an impact on the growing interest⁶ on the potential role of galectin-3 as a therapeutic target? Although the absence of a role for galectin-3 in the pathogenesis of cardiac dysfunction and fibrosis observed in our

Supported by NIH grants R01 HL76246 and R01 HL85440.

Disclosures: None declared.

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investigation somewhat weakens the conceptual basis underpinning the use of galectin-3 as a therapeutic target, the findings should be cautiously interpreted.

The goal of a well-designed loss-of-function study in an animal model is to dissect the role of a specific mediator in a relevant pathophysiologic response. Our aim was to gain pathophysiologic insights and not to test the effectiveness of a therapeutic strategy in human patients. To this end, we used age- and sex-matched mice with identical genetic backgrounds in an established and standardized experimental model that recapitulates certain pathophysiological aspects of the disease. Thus, experimental groups of mice differ only in the presence or absence of the mediator. In contrast, in the clinical setting, most diseases are characterized by marked pathophysiological heterogeneity, as patients have different genetic backgrounds and phenotypic characteristics, and they exhibit pathophysiologically distinct forms of the disease. Thus, animal models should be optimally used as tools for mechanistic dissection and not as predictors of therapeutic effectiveness.⁷

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<http://dx.doi.org/10.1016/j.ajpath.2016.06.001>